

## Remarks

### Amendments to the claims

Claim 5 has been amended to correct a typographical error in the spelling of the word "periosteal". Claim 5 has also been amended to include osteocytes among the cells that can be encapsulated in the scaffold. Support for this addition is found on page 31, lines 19-21.

Support for new claim 19 is found throughout the specification, which describes encapsulation of chondrocytes in detail. See, e.g., page 23, line 21 – page 26, line 21.

Support for new claim 20 is found at page 23, line 22, of the specification.

Support for new claim 21 is found at page 8, lines 7-10, of the specification.

Support for new claim 22 is found at page 15, lines 10-12, indicating that secretion of extracellular matrix components increased the strength of the scaffold. As is well known to one of ordinary skill in the art, equilibrium compression modulus is but one of a multitude of possible ways in which strength can be measured.

Support for new claim 23 is found at page 2, lines 2 – 4, of the specification.

Support for new claim 24 is found at a variety of places in the specification, including at page 15, lines 10-12, and at page 26, lines 4-7, indicating that the equilibrium compression modulus of a scaffold without cells is only 0.5% and that the equilibrium compression modulus of the macroscopic scaffold with cells is at least 50-fold as high at day 28.

Support for new claim 25 is also found at page 26, lines 4-7.

Support for new claim 26 is found at page 16, lines 7-9, indicating that the macroscopic scaffolds of the invention are useful for repairing defects in soft tissues that have equilibrium compression moduli much lower than that of cartilage, e.g., 1.51 and 1.99, which, Applicants submit, is approximately 2.

Support for new claim 27 is found at page 7, line 24, through page 8, line 3, and at page 26, lines 4-7.

Support for new claim 28 is found at page 5, line 19, indicating that in a preferred embodiment of the invention the cells are autologous or allogeneic.

Support for new claim 29 is found at page 9, lines 3 – 5.

Support for new claim 30 is found at page 8, lines 11 – 19.

Support for new claim 31 is found at page 26, lines 14-16, indicating formation of a macroscopic scaffold using a cell density of approximately  $0.5 \times 10^6$  cells/ml and at page 24, line 2.

Support for new claim 32 is found at page 26, lines 18-20, indicating that cells divided within the scaffold.

#### Rejections under 35 U.S.C. § 112

Claims 1-8 stand rejected under 35 U.S.C. § 112 as being indefinite.

The Examiner has asserted that claim 1 is indefinite in that it is unclear as to whether the scaffold is formed by the peptides self-assembling or whether the peptides are only a component of the scaffold. Claim 1 has been amended to clearly indicate that the scaffold is formed by self-assembly of the peptides to encapsulate cells, as stated on page 2, lines 19-20. However, Applicants note that this does not exclude the presence of other components on or in the scaffold, provided that such components do not disrupt the ability of the peptides to self-assemble.

The Examiner has asserted that claim 2 is indefinite in that the term "chemoattractant" does not appear to have an art-recognized meaning. Applicants respectfully disagree. According to the Collins English Dictionary, Harper Collins 2000, as displayed online at <http://www.wordreference.com/english/definition.asp?en=chemoattractant>, a "chemoattractant" is defined as "a chemical substance that provokes chemotaxis, esp. one that causes a bacterium to move in the direction in which its concentration is increasing". "Chemotaxis" is in turn defined as "the movement of a microorganism or cell in response to a chemical stimulus". Furthermore, the Dictionary of Cell Biology, Academic Press, 3<sup>rd</sup> edition, 1999, available online at <http://www.mblab.gla.ac.uk/~julian/Dict.html>, defines chemoattractant as "a substance that elicits the accumulation of cells". A search of the National Library of Medicine's PubMed database of biological and medical journals performed on October 5, 2003, identified 27,646 articles that use the term "chemoattractant". Page 1 of this search is attached as Exhibit A. When the search was limited to articles predating the filing date of the instant application, 21,090 articles were identified. Page 1 of this search is attached as Exhibit B. Many of these articles describe specific assays used to demonstrate that a particular substance behaves as a chemoattractant. It is thus evident that the term "chemoattractant" has a recognized meaning in the English language, and this meaning is consistent with its meaning in the art. It is further

evident that one of ordinary skill in the art would readily understand the meaning and scope of Applicants' claims and could readily turn to the literature to determine whether any particular compound was a chemoattractant. As the Federal Circuit has stated, claims need only "reasonably apprise those skilled in the art" as to their scope and be "as precise as the subject matter permits". *Hybritech Inc., v. Monoclonal Antibodies, Inc.*, 802 F.2d. 1367, 231 (Fed. Cir. 1987).

The Examiner has asserted that claim 7 is indefinite in that the term "equilibrium compression modulus" does not appear to have an art-recognized meaning. Applicants respectfully disagree. The Examiner's attention is drawn to the review article attached as Exhibit C (Mow, V.C., and Guo, E., "Mechano-Electrochemical Properties of Articular Cartilage: Their Inhomogeneities and Anisotropies", *Annual Review of Biomedical Engineering*, 4: 175-209 (2002)). On page 180, which is tabbed for the Examiner's convenience, it is stated that, "In a stress-relaxation or creep test under confined compression, characterized by one-dimensional motion but multidimensional loading (due to the constraining sidewalls), two intrinsic material properties can be determined: (a) the equilibrium confined compression aggregate modulus ( $H_A$ , measured in units of MPa or  $10^6$  Pa)..." On p. 26, lines 1 – 7, of the instant application, the procedure used to determine the equilibrium compression modulus is described as follows: "For mechanical testing of the peptide scaffold, a 6 mm diameter by 1.5 mm thick cylindrical plug was taken from the scaffold at day 28. The plug was subjected to various levels of compression and the level of stress was measured, as described previously (Buschman *et al.*, *supra*). (Figs. 6 and 7). Based on these results, the equilibrium modulus for the scaffold containing chondrocytes was 27 kPa..." In the Buschman reference (Buschman *et al.*, *J. Cell Science*, 108: 1497-1508 (1995), attached as Exhibit D), the method employed by Applicants to determine the equilibrium compression modulus is described at p. 1499, "Physical characterization". As stated there, "disks were tested in confined compression geometry using the mechanical spectrometer. The disk was placed in a tight-fitting confining cylindrical well..." On page 1500, under "Physical properties", Buschman, *et al.*, state that they obtained, "The confined compression equilibrium modulus,  $H_A$  (a measure of the static equilibrium stiffness of the tissue)..." It is thus evident that one of ordinary skill in the art would readily understand the term "equilibrium compression modulus" as found in claim 7, to mean the intrinsic material property  $H_A$ , which may also be

referred to as the "aggregate modulus" or "equilibrium confined compression aggregate modulus".

The Examiner has asserted that claim 8 is confusing in that it requires at least 60% of the encapsulated cells to be in cell-cell contact with another encapsulated cell or another cell outside the scaffold without setting forth conditions that result in the contact with another encapsulated cell or cell outside the scaffold. Claim 8 describes a composition of matter, namely a scaffold that encapsulates cells. Applicants submit that there is no need that the conditions necessary to produce or achieve the structure be set forth in the claim itself. Such a requirement would be akin to requiring an inventor of a new chemical compound to set forth the synthetic route used to produce the compound in the claim itself. Such a requirement would unduly limit the scope of the claim. In addition, Applicants submit that certain of the conditions that may result in a scaffold having at least 60% of the encapsulated cells in cell-cell contact with another encapsulated cell are evident from the specification. As described on page 3, lines 20-21, the encapsulated cells are present in the macroscopic scaffold in a three-dimensional arrangement and, in a preferred embodiment, are "substantially uniformly distributed" (page 6, lines 16-17), which term is further defined at page 11, lines 15-21. Therefore, whether at least 60% of the encapsulated cells are in cell-cell contact with another encapsulated cell is dependent in part on the density of cells in the scaffold, which is in turn dependent on the concentration of cells in the suspension used to form the scaffold and on the extent of cell proliferation that occurs after encapsulation of the cells. The specification describes forming scaffolds using different initial concentrations of cells (see page 24, line 3 and page 26, lines 14-15). The specification further describes that chondrocytes proliferated after encapsulation (see page 26, lines 20-21). Thus the initial cell density and the extent of proliferation are parameters which, together with factors such as the extension of cell processes, influence the extent of cell-cell contact within the scaffold.

Claim 8 has been amended to remove the portion referring to contact with cells outside the scaffold as it is evident that whether at least 60% of the encapsulated cells are in cell-cell contact with another cell outside the scaffold depends in part on the particular environment in which the scaffold is placed rather than depending entirely on the scaffold and the encapsulated cells.

#### Rejections under 35 U.S.C. § 102

Claims 1, 3, 5, 6, and 7 stand rejected under 35 U.S.C. § 102(a) as being anticipated by Kisiday, *et al.* Applicants respectfully traverse this rejection. Kisiday, *et al.* lists the inventors of the instant application as authors. In addition, Kisiday, *et al.*, lists M. Jin, B. Kurz, and H. Hung as authors. Enclosed is a Declaration of Dr. Kisiday, Dr. Grodzinsky, and Dr. Zhang under 37 C.F.R. § 1.132, stating that M. Jin, B. Kurz, and H. Hung are not inventors of the subject matter claimed in this application. As described on page 2 of the Declaration, the inventors communicated the subject matter of the invention to M. Jin, B. Kurz, and H. Hung, who provided technical assistance under the inventors' direction. Therefore, Kisiday, *et al.* is no longer a reference under 35 U.S.C. § 102(a). See MPEP § 715.

Applicants therefore request that the rejection of claims 1, 3, 5, 6, and 7 over Kisiday, *et al.* be withdrawn.

#### Rejections under 35 U.S.C. § 103

Claims 2 and 8 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Kisiday *et al.* in view of Hubbell (6,129,761). Applicants respectfully traverse this rejection. As noted above, enclosed is a declaration of Dr. Kisiday, Dr. Grodzinsky, and Dr. Zhang stating that M. Jin, B. Kurz, and H. Hung are not inventors of the subject matter claimed in this application. Therefore, Kisiday, *et al.* is no longer a reference for purposes of establishing obviousness under 35 U.S.C. § 103.

Claims 2 and 8 are patentable because Kisiday, *et al.* is not a reference, and Hubbell does not disclose or suggest the claimed invention. Hubbell says absolutely nothing about using self-assembling peptide gels to encapsulate cells. Thus Hubbell taken alone cannot render any aspect of the invention obvious.

Claim 4 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Kisiday, *et al.* in view of Holmes *et al.* (PNAS 2000), hereinafter Holmes (PNAS). Claim 4 is patentable because Kisiday, *et al.* is not a reference, and, as pointed out by the Examiner, Holmes (PNAS) discloses attaching neurons to a self-assembling peptide scaffold and growing the neurons. The neurons of Holmes (PNAS) are placed on the surface of the scaffold after self-assembly of the peptides rather than being encapsulated within the scaffold. Holmes (PNAS) does not disclose or suggest methods or procedures for achieving encapsulation of neurons, and it provides no

evidence to suggest that neurons could survive or grow when encapsulated. Reading Holmes (PNAS) would not provide one of ordinary skill in the art with the knowledge of how to encapsulate cells, a process that required additional inventive steps disclosed in the instant application. Thus Holmes (PNAS) taken alone cannot render any aspect of the invention obvious.

Claims 1-3 and 5-8 stand rejected under 35 U.S.C. § 103(a) as being unpatentable over Holmes *et al.* (5,955,343), hereinafter Holmes, or Zhang *et al.* (Biomaterials 1995), hereinafter Zhang, in view of Hubbell (6,129,761), hereinafter Hubbell, and if necessary in further view of Kisiday, *et al.* As discussed above, Kisiday, *et al.* is not a reference, thus this response will address the rejection as based on Holmes, Zhang, and Hubbell.

Applicants respectfully submit that Holmes, Zhang, and Hubbell do not render the invention of claims 1-3 and 5-8 obvious for the reasons set forth below. First, it is well established that an obviousness determination must not be based on hindsight. As pointed out by the Examiner, Holmes and Zhang disclose culturing cells on a membrane or matrix formed by self-assembly of peptides. Hubbell purports to disclose that cells encapsulated in any of a variety of previously known slowly polymerizing hydrogels are useful for implanting. Among the materials discussed extensively by Hubbell are a number of polymers including carbohydrates such as alginate and various polysaccharides. Hubbell briefly mentions that, "Other materials which may be utilized include proteins such as fibrin, collagen, and gelatin." (col. 8, lines 61-62). This teaching does not suggest the use of peptides for formation of hydrogels, since all the examples of proteins provided by Hubbell are large proteins that naturally occur within the body of mammalian organisms. The only method contemplated by Hubbell to form a hydrogel using amino acid polymers is enzymatic cross-linking, rather than self-assembly (col. 9, lines 61-62). In particular, Hubbell does not disclose or suggest the use of self-assembling peptides to form hydrogels for encapsulation of cells or disclose methods whereby peptides could be used to form a hydrogel. Hubbell's extensive teachings regarding the utility of carbohydrate-based structures for encapsulating cells does not suggest that cells could be successfully encapsulated in materials with very different properties such as peptides and does not suggest how such a result could be achieved. Such teachings are to be found within the instant application, which describes methods for practicing the invention as claimed. As the Federal Circuit has stated on numerous

occasions, "Obviousness may not be established using hindsight or in view of the teachings or suggestions of the inventor." *Para Ordnance Manufacturing, Inc. v. SGS Importers International, Inc.*, 73 F.3d 1085 (Fed. Cir. 1995).

In particular, the combination of Holmes, Zhang, and Hubbell does not enable the instant invention. There is no teaching in Hubbell, Holmes, or Zhang, of the proper way to combine the references so as to achieve the intended useful result disclosed by Hubbell, i.e., a scaffold suitable for implantation into a subject for therapeutic purposes. As the Federal Circuit has recently reaffirmed, " 'In order to render a claimed apparatus or method obvious, the prior art must enable one skilled in the art to make and use the apparatus or method.' " *Motorola, Inc. v. Interdigital Technology Corp.*, 121 F.3d 1461 (Fed. Cir. 1997), quoting from *Beckman Instruments, Inc. v. LKB Produktur AB*, 892 F.2d 1547 (Fed. Cir. 1989). Furthermore, "In holding an invention obvious in view of a combination of references, there must be some suggestion, motivation, or teaching in the prior art that would have led a person of ordinary skill in the art to select the references and combine them in the way that would produce the claimed invention." *Karsten Manufacturing Corp. v. Cleveland Golf Co.*, 242 F.3d 1376 (Fed. Cir. 2000). (emphasis added). Even if the teachings of Hubbell could be said to include use of peptides to form hydrogels encapsulating cells, none of the references disclose how to encapsulate cells in a macroscopic scaffold formed by self-assembly of peptides, said cells being present in the macroscopic scaffold in a three-dimensional arrangement, as required by the instant claims.

Developing the method to encapsulate cells in a macroscopic scaffold formed by peptide self-assembly such that the cells are present in a three-dimensional arrangement required additional inventive steps not disclosed or suggested by any of the references cited by the Examiner. For example, as described on page 2, lines 13-15, of the instant application, the method "involves incubating peptides and living cells in an aqueous solution having an iso-osmotic solute under conditions that do not allow the peptides to substantially self-assemble." This step is followed by addition of an electrolyte, which allows self-assembly to proceed. (See p. 2, lines 18-21). However, Holmes and Zhang disclose a method in which peptides are added to tissue culture medium containing cells, which resulted in the formation of membranes that did not encapsulate the cells (see, e.g., col. 3, lines 32-35 of Holmes). Holmes further discloses that various monovalent cations can induce membrane formation (see, e.g., col. 7, lines 50-60). Holmes discloses that cells attach to the membranes and that they are useful for culturing cell

monolayers (see, e.g., col. 11, lines 32-43). Nowhere do Holmes or Zhang discuss how their teachings might be modified to encapsulate cells. Thus the teaching of Holmes and Zhang do not enable the instant invention.

In describing how to produce a hydrogel encapsulating cells, Hubbell states that, "Preferably the polymer is dissolved in an aqueous solution, preferably a 0.1 M potassium phosphate solution, at physiological pH, to a concentration forming a polymeric hydrogel... The isolated cells are suspended in the polymer solution..." Simply reading this cursory description would not apprise one of ordinary skill in the art of the method of maintaining the peptides and living cells together in an iso-osmotic solute under conditions that do not allow the peptides to substantially self-assemble and then exposing the solution to an electrolyte to initiate self-assembly. It is evident that were one to follow the teachings of Hubbell in an attempt to encapsulate cells using self-assembling peptides, one would dissolve the peptides in an aqueous solution under conditions (e.g., 0.1 M potassium phosphate) under which self-assembly might actually begin and then add cells. While such an approach might work and is not excluded in the practice of the instant invention, in the absence of evidence to show that practicing this method would result in encapsulation of cells in a three-dimensional arrangement, it cannot be said that the method enables formation of a scaffold having the features required by the instant claims. In particular, it appears quite possible that adding cells to a solution in which peptide self-assembly has already started to occur would result in a local accumulation of cells at the edge of the scaffold rather than a three-dimensional arrangement. Thus neither Holmes and Zhang nor Hubbell enable a method by which cells can be encapsulated in a scaffold formed by self-assembly of peptides.

Even if enablement existed, the references provide no reasonable expectation of success that cells encapsulated in a self-assembling peptide hydrogel would survive, let alone produce a structure useful for implanting. As the Federal Circuit has stated, "'For a [prior art reference that arguably suggests a claimed invention] to render the claimed invention obvious, there must have been, at the time the invention was made, a reasonable expectation of success..." *Life Technologies, Inc. v. Clontech Laboratories, Inc.*, 224 F. 3d 1320 (Fed. Cir. 2000), citing *Micro Chem., Inc. v. Great Plains Chem. Co.*, 103 F.3d 1538, 1547 (Fed. Cir. 1997) and *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988). It is questionable whether Hubbell would provide one of ordinary skill in the art with a reasonable expectation of success for the encapsulation of

cells within any of the polymeric hydrogels he mentions, let alone hydrogels formed from novel materials such as the peptides of the instant invention. While Hubbell might teach how to encapsulate cells within the materials he describes, he presents absolutely no data or working examples demonstrating survival of the cells. Absent survival, the encapsulation of cells envisioned by Hubbell cannot be said to be successful since the purposes envisioned for the encapsulated cells (implantation into a subject for therapeutic purposes) require survival.

Holmes presents data showing that neurons can attach to the surface of a two-dimensional membrane formed by self-assembly of amphiphilic peptides and that the membranes are not toxic to these cells (col. 10, lines 45-50). Zhang reports similar results for a variety of other cell types. However, the mere fact that cells can grow on the surface of a membrane formed by self-assembly of amphiphilic peptides is not sufficient to provide a reasonable expectation that these cells, or any cells, would be able to survive under the very different conditions of encapsulation within a scaffold formed by self-assembly of the peptides. Furthermore, Holmes and Zhang provide no evidence to suggest that cells could survive the relatively rapid changes in the extracellular environment associated with self-assembly. When cells are grown on a membrane they are bathed in medium, and nutrients and waste products can freely diffuse. Furthermore, only one surface of the cell is in contact with the hydrogel material. Until Applicants successfully encapsulated cells and measured their survival within the scaffold (see page 24, lines 15-20), there was no reason to believe that such survival would be achieved. Indeed only 75% of the cells did survive after 24 hours, further indicating that it was not obvious that cells would survive encapsulation in self-assembling peptide hydrogels. Thus one of ordinary skill in the art, reading Hubbell, Holmes, and Zhang, would not be provided with a reasonable expectation that cells encapsulated in scaffolds formed by self-assembly of amphiphilic peptides would survive to form a structure useful for implantation. At the very most, one of ordinary skill in the art, reading the cited references would have found it obvious to try to encapsulate cells in such a material. However, the Federal Circuit has consistently stated that, “ ‘[O]bvious to try’ is not the standard.” *Eclochem, Inc. v. Southern California Edison Co.*, 227 F.3d 1361 (Fed. Cir. 2000).

In summary, the combination of Holmes, Zhang, and Hubbell does not render the instant invention obvious because (i) the combination does not teach the invention without the use of hindsight and the addition of inventive steps found only in the instant application, and is

therefore not enabling; and (ii) the combination of references does not provide a reasonable expectation of success in achieving a useful result.

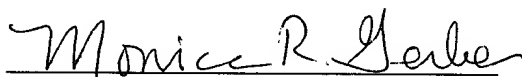
Claim 4 stands rejected under 35 U.S.C. § 103(a) as being unpatentable over Holmes, *et al.* (5,955,343) or Zhang, *et al.* (*Biomaterials* 1995) in view of Hubbell (6,129,761), Kisiday, *et al.*, and further in view of Holmes, *et al.* (PNAS 2000). As pointed out above, Kisiday, *et al.* is not a reference, thus this response will address the rejection as based on the Holmes '343 patent, Zhang, *et al.*, the '761 patent, and Holmes. Applicants respectfully traverse this rejection for all the reasons set forth in their traversal of the rejection of claims 1-3 and 5-8. Claim 4 limits the scope of claim 1 by requiring that the cells encapsulated by the self-assembly of amphiphilic peptides are neurons. As described above, the combination of Hubbell, Zhang, and the '343 patent of Holmes do not render the instant invention obvious for any cell type. The addition of Holmes (PNAS 2000) does not go further towards establishing obviousness since the results it presents, as is the case for the Holmes '343 patent and the Zhang paper, are confined to a demonstration of cell behavior on the surface of a scaffold formed by self-assembly of amphiphilic peptides. The results reported in Holmes (PNAS 2000) are significant in that they show that the surface of the scaffold is a permissive substrate for synapse formation, but they provide no evidence that similar results would be obtained when neurons are encapsulated within such a scaffold. Thus Applicants submit that the addition of Holmes (PNAS 2000) to the other references does not render claim 4 obvious.

In conclusion, in view of the amendments and remarks presented herein, none of the cited art anticipates any of the claims pending in the instant application nor renders them obvious. Applicants therefore respectfully submit that the present case is in condition for allowance. A Notice to that effect is respectfully requested.

If, at any time, it appears that a phone discussion would be helpful, the undersigned would greatly appreciate the opportunity to discuss such issues at the Examiner's convenience. The undersigned can be contacted at (617) 248-5000 or (617) 248-5071 (direct dial).

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Respectfully submitted,

A handwritten signature in cursive script that reads "Monica R. Gerber". The signature is written in dark ink and is positioned above the printed name.

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Date: October 8, 2003

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